Stem Cell Research 16 (2016) 287–289

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Stem Cell Line

Generation of Hermansky Pudlak syndrome type 2 (HPS2) induced pluripotent stem cells (iPSCs)

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ARTICLE INFO

Article history:
Received 5 January 2016
Accepted 12 January 2016
Available online 14 January 2016

ABSTRACT

Hermansky–Pudlak syndrome type 2 (HPS2) is a rare autosomal recessive disorder resulting from functional mutations in the adaptor-related protein complex 3, beta 1 subunit (AP3B1) gene. This gene plays a role in organelle biogenesis associated with melanosomes, platelet dense granules, and lysosomes. Here we describe the generation of an HPS2 iPS cell line (*CHOPHPS2*) using a Cre-excisable polycistronic STEMCCA lentivirus. This line was derived from human fibroblasts isolated from a patient carrying two mutations in the AP3B1 gene. The patient presented with severe neutropenia, ocular albinism, interstitial pulmonary fibrosis, hemorrhagic diathesis, and an absence of platelet-dense granules.

Resource table: *CHOPHPS2*

Name of stem cell construct	*CHOPHPS2*
Institution	The Children's Hospital of Philadelphia (CHOP)
Person who created resource	Lin Lu
Contact person and email	Deborah L. French, frenchd@email.chop.edu
Date archived/stock date	2012
Origin	Human skin fibroblasts (GM17890, Coriell Cell Repositories)
Type of resource	Biological reagent: iPSC derived from human skin fibroblasts
Subtype	Cell Line
Key transcription factors	Oct4, Sox2, Klf4, c-Myc
Authentication	Identity and purity of cell line confirmed (Fig. 1)
Link to related literature	Huizing et al., 2002
Information in public databases	N/A

Resource details

Human skin fibroblasts isolated from a 4-year-old male HPS2 patient were obtained from the Coriell Cell Repositories (Camden, NJ). The parental line, GM17890, carries a compound heterozygous mutation in the AP3B1 gene: C1578T (R508X) in exon 15 and G2028T (E659X) in exon 18. The cells were transduced with a STEMCCA Cre-excisable Constitutive Polycistronic lentivirus expressing Oct4, Klf4, Sox2, and c-myc. Cre-mediated excision of the vector following reprogramming was demonstrated (Fig. 1A). The mutation was validated by DNA sequencing and quantitative PCR (qPCR) (Fig. 1B). The cells exhibited a normal karyotype (46, XY) upon G-band analysis (Fig. 1C).

Pluripotency was verified by gene expression of stem cell markers DMNT3B, REX1, ABCG2, OCT4, NANOG, and SOX2 using qPCR (Fig. 1E). In addition, expression of pluripotent surface markers at single cell resolution were confirmed by flow cytometry (Fig. 1D). Differentiation capacity into three germ layers was confirmed by in vivo teratoma formation (Fig. 1F).

Materials and methods

Cell culture and iPSC reprogramming

Human HPS2 fibroblast line, GM17890, was cultured in IMDM (Mediatech) containing 10% FBS at 37 °C and 5% CO_2 in a humidified incubator. STEMCCA lentiviral vector was produced, and cells were infected as previously described (Somers et al., 2010). The STEMCCA Cre-Excisable Constitutive Polycistronic viral vector used for reprogramming encoded Oct4, Klf4, Sox2, and c-myc. Human fibroblasts were incubated with 5–10 μl STEMCCA virus plus 5 μg/ml of polybrene in fibroblast medium. Six days after infection, cells were replated on 100-mm dishes containing irradiated mouse embryonic fibroblasts (MEFs). The cells were maintained in IMDM with 10% FBS, NEAA (1%), glutamine (1%), penicillin/streptomycin (1%), 50 μg/ml ascorbic acid, and 4 ng/ml bFGF for 4 days. Thereafter, cells were maintained in human embryonic stem cell medium (HES) containing 4 ng/mL of bFGF. The medium was replenished every 2–3 days for 3 weeks until uniform colonies were generated. The iPSC colonies were mechanically isolated and expanded on MEFs.

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http://dx.doi.org/10.1016/j.scr.2016.01.015

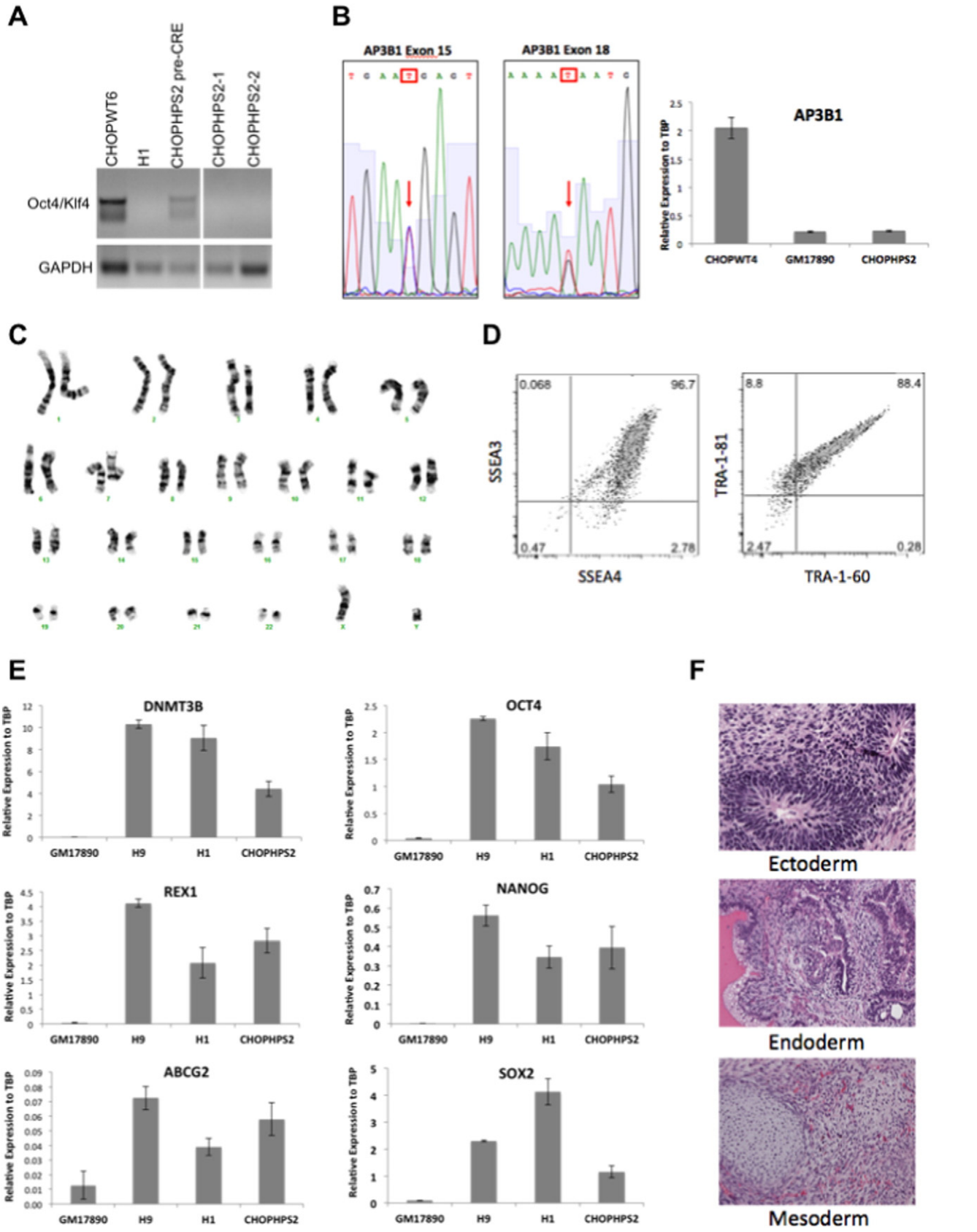

Fig. 1. Generation and characterization of CHOPHPS2. (A) Cre-mediated excision of the lentiviral vector in three clones of the CHOPHPS2 iPSC line. Cells from a non-excised lentiviral iPSC line were used as a positive control. (B) Validation of the HPS2 mutation by DNA sequencing of the AP3B1 gene (exons 15 and 18) and qPCR measurement of gene expression. (C) Karyotype analysis of CHOPHPS2. (D) Flow cytometry profiles of surface markers on undifferentiated cells: SSEA3, SSEA4, TRA-1-60, and TRA-1-81. (E) Quantitative PCR of relative gene expression of pluripotency genes compared to two embryonic stem cell lines (H9 and H1) and the parental fibroblast line (GM17890). (F) H&E staining of teratoma sections identifying the three germ layers.

Cre-mediated STEMCCA excision

Transgene removal was performed as previously described (Somers et al., 2010). Briefly, iPSCs were transfected with 2 μg/well of pHAGE2-Cre-IRES-PuroR plasmid DNA using Hela Monster transfection reagent (Mirus, Madison, Wi) according to the manufacturer's instructions. Cre-excised iPSC colonies were isolated by growth in puromycin (1.2 μg/ml) selection medium 24 h post-transfection for 48 h. Genomic DNA was isolated using the DNeasy Blood & Tissue kit (Qiagen, Valencia, CA) from each subclone after 2–3 weeks in culture and screened for hSTEMCCA transgenes using the following primers: OCT4-Tg-F: $^{5'}$GGT GCG CCA GTA AAG CAG ACA TTA AAC$^{3'}$; KLF4-Tg-R: $^{5'}$CAG ACG CGA ACG TGG AGA AAG A$^{3'}$ and GAPDH-F: $^{5'}$GTG GAC CTG ACC TGC CGT CT$^{3'}$; GAPDH-R: $^{5'}$GGA GGA GTG GGT GTC GCT GT$^{3'}$.

Mutation verification

PCR amplification was performed on genomic DNA isolated from iPSCs using the referenced primer sets: AP3B1 exon 15, $^{5'}$ATAGTTAA AACCTTTTCTATG$^{3'}$ and $^{5'}$TGATCAGAGTGAAGAGGAAAG$^{3'}$ AP3B1 exon 18, $^{5'}$AGCTTGCTTTTGAATTCAGT$^{3'}$ and $^{5'}$TCTGAGATATAGAATGTAATC$^{3'}$ (Huizing et al., 2002). Products were sequenced by the CHOP NAPCore facility. Expression of the mutated AP3B1 gene was shown by qPCR using the following primers: $^{5'}$TTGGTAGCTGGCAGTGTTGTGATG$^{3'}$ and $^{5'}$CCGAGCATATCGAGTTAGCATGTGGA$^{3'}$.

Karyotype analysis

Chromosomal G-band analyses were performed in the Genomics Core Laboratory (CHOP).

Flow cytometry analysis

Expression of pluripotency markers were evaluated by flow cytometry using the following antibodies: Alexa-Fluor®-647 α-human SSEA4 (1:400) and Tra-1-81 (1:200); Alexa-Fluor®-488 SSEA3 (1:200) and Tra-1-60 (1:200) (BioLegend). Accutase-dissociated single cells were analyzed using a FACSCanto flow cytometer (BD Biosciences) and the FlowJo software program (Tree Star, CA).

RT-PCR and quantitative PCR

RNA was isolated from the CHOPHPS2 iPSCs using the RNeasy micro kit (Qiagen) according to the manufacturer's instructions. Reverse transcription was performed on total RNA (500 ng) using random hexamers and Superscript III Reverse Transcriptase (Life Technologies). RT-qPCR was performed on a LightCycler-480II (Roche, IN). All experiments were performed in triplicate using SYBR-Green qPCR supermix (Roche) according to the manufacturer's instructions. Primers for endogenous genes (DMNT3B, REX1, ABCG2, OCT4, NANOG, and SOX2) were prepared as previously described (Mills et al., 2013). Human genomic DNA, diluted in 10-fold increments from 1 to 100 ng/μl, was used to generate a standard curve to determine PCR efficiency and relative gene expression compared to the housekeeping gene TBP (TATA binding box protein).

In vivo teratoma formation

CHOPHPS2 cells were cultured in feeder-free conditions and dissociated using trypsin. Single cells (~10^6) were resuspended in medium containing growth-factor reduced matrigel (100 μl of 1:3 dilution in IMDM) (Corning), and injected subcutaneously in the neck of NOD/SCID mice. After 6–8 weeks, teratomas were excised and fixed in 4% phosphate-buffered formalin for 24 h. The fixed samples were paraffin embedded and hematoxylin and eosin (H&E) stained for histological analysis. This work was approved by the IACUC committee at CHOP.

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